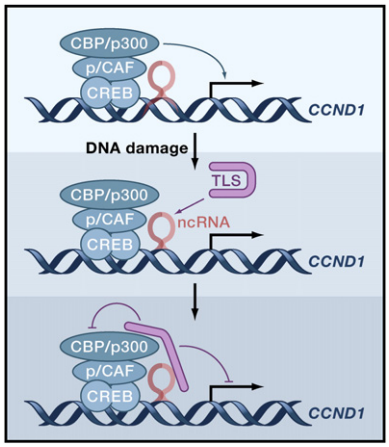
Molecular Biology Select

Cell

Progress toward understanding the function and regulation of noncoding RNAs is the topic of this issue's Molecular Biology Select. Recent reports provide mechanistic insight into the regulation of transcription by noncoding RNAs found at gene promoters. Other findings reveal a new mechanism for the regulation of attenuated noncoding transcripts, provide evidence for the involvement an antisense transcript in Alzheimer's disease, and shed light on the evolutionary conservation of imprinting mechanisms in mammals.



DNA damage signals induce expression of noncoding RNAs from the *CCND1* promoter region that recruit and induce a conformation change in TLS, which inhibits CBP and p300, leading to transcriptional repression. Figure courtesy of C. Glass.

Noncoding RNAs Build a Bridge to Transcription's End

Cyclin D1 is a cell cycle regulator that is repressed by DNA damage to prevent cell cycle progression while DNA lesions are being repaired. Wang et al. (2008) have recently shown that DNA damage signals enhance the expression of noncoding RNAs that are tethered to the 5′ regulatory region of the cyclin D1 gene. These noncoding RNAs recruit the RNA binding protein translocated in liposarcoma (TLS), which in turn binds and inhibits the histone acetyltransferases CREB-binding protein (CBP) and p300. The resulting suppression of local histone acetylation inhibits cyclin D1 transcription. Remarkably, this entire process is RNA dependent, indicating that the noncoding RNA is an allosteric regulator of TLS. These findings reveal a new means by which transcription can be regulated in response to cellular signals and a new function for noncoding RNAs. Future efforts will determine the chain of signals that link DNA damage to the expression of the noncoding RNAs and may reveal whether noncoding RNAs are expressed in the 5′ regulatory regions of other genes whose expression is repressed by DNA damage signals.

*X. Wang et al. (2008). Nature **454**, 126–130.*

A Hidden Meaning behind a Pol II Initiation Ritual

For some genes, the selection of the start site of transcription is an important feature of their regulation, with some start sites leading to attenuated transcripts that are targeted for degradation. Kuehner and Brow (2008) now show that the concentration of nucleotides can regulate which transcription start site is selected by RNA polymerase II (pol II). Although the potential functional consequences of this type of regulation are numerous, the example provided by the authors is a compelling one.They studied transcription of the yeast gene *inosine monophosphate dehydrogenase 2* (*IMD2*), which encodes a key enzyme in guanosine triphosphate (GTP) biosynthesis. They observe that when the GTP concentration is high, upstream guanines are favored as the start sites for transcription. This leads to the production of attenuated noncoding transcripts because of the presence of a terminator sequence recognized by the helicase Sen-1 via its associated RNA-binding proteins. When GTP levels are low, this terminator sequence is bypassed, and pol II instead chooses a downstream adenine as the start site. This leads to transcription through the coding region of the *IMD2* gene, enhanced Imd2 protein expression, and GTP biosynthesis. The new study uncovers a clever means by which the cell maintains nucleotide homeostasis. The authors also provide evidence that the effect of nucleotide concentration appears to be mediated through the Rpb1 subunit of pol II. Whether other genes are similarly regulated by nucleotide concentration or whether this pathway is present in other organisms awaits further exploration.

*J.N. Kuehner and D.A. Brow (2008). Mol. Cell **31**, 201–211.*

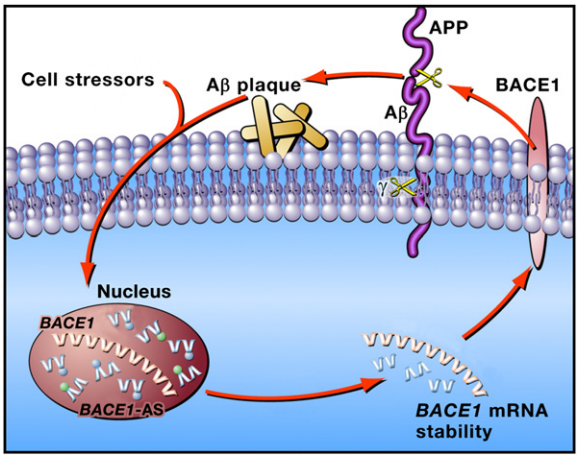
Making Sense of Antisense

Previous work has indicated that synthetic small interfering RNAs directed to promoters can alter gene expression in mammalian cells. Argonaute proteins may be involved in this process. However, given that complexes of Argonautes and small RNAs normally bind to target mRNA sequences, it has been unclear how the Argonautes would be targeted to a region of a gene that is not known to be transcribed. In their recent work, Schwartz et al. (2008) report data that may reconcile these results. By examining transcription at the progesterone receptor gene locus in cultured human cancer cells, the authors uncovered the existence of endogenous antisense transcripts that overlap with the promoter region of the gene. They show that the synthetic small RNAs are recruited to the gene promoter through the recognition of the antisense transcript and that a decrease in the expression of the antisense transcript impairs the ability of promoter-directed small RNAs to alter expression of the progesterone receptor gene. For small RNAs that activate gene expression, their recruitment to the promoter shifts the

localization of heterogeneous nuclear ribonucleoprotein K (hnRNP-k) from DNA to RNA and leads to a decrease in heterochromatin protein 1γ (HP1γ) at the promoter. Homologs of HP1γ in yeast are integral to RNA-induced transcriptional silencing (RITS), and future work may further characterize the similarities and differences between RITS and the action of synthetic promoter-directed small RNAs. It is also an open question as to whether endogenous small RNAs in mammalian cells, rather than synthetic RNAs, might be found that similarly target promoter sequences.
J.C. Schwartz et al. (2008). Nat. Struct. Mol. Biol. Published online July 6, 2008. 10.1038/nsmb.1444.

In Alzheimer's Disease, *BACE* Is Mistuned

The protease BACE1 (also known as β-Secretase) is involved in the processing of amyloid precursor protein and is critical for generation of the Aβ1-42 peptide implicated in the pathogenesis of Alzheimer's disease. Faghihi et al. (2008) now provide evidence that *BACE1* expression is regulated by an endogenous *BACE1* antisense transcript. The antisense transcript encompasses a large segment of the *BACE1* locus, and, like the sense transcript, it is spliced and polyadenylated, yet no coding region can be found within it. Interestingly, knockdown of the antisense transcript in cultured human cell lines and the brain of living mice with short interfering RNAs led to a simultaneous decrease in the expression of the sense transcript, suggesting that the antisense transcript is a positive regulator of *BACE1* expression. Likewise, knockdown of the antisense transcript also decreased the production of Aβ peptide in human embryonic kidney cells bearing a mutation in the amyloid precursor protein that has been linked to early-onset Alzheimer's disease. The authors show that the sense and antisense transcript can form a duplex that stabilizes the sense transcript. They also show that the antisense transcript is elevated in the brains of Alzheimer's disease patients, suggesting a possible involvement in disease pathogenesis. Future work might further explore how the expression of the antisense transcript is regulated longitudinally in vivo. As a step in this direction, the authors show that environmental stressors, such as serum deprivation or high temperatures, can elevate the expression of the antisense transcript in cultured cells.
M.A. Faghihi et al. (2008). Nat. Med. ***14****, 723–730.*



Proposed role of the *BACE1* antisense transcript (*BACE1-AS*) in plaque buildup in Alzheimer's disease. Figure courtesy of C. Wahlestedt.

A tammar wallaby with a large pouch young. Photo courtesy of G. Shaw.

H19 Noncoding RNA Leaves a Lasting Evolutionary Imprint

Genomic imprinting ensures the expression of a copy of a gene in a manner specified by the parent of origin. As a topic of major interest in human biology, it is worth considering the fact that genomic imprinting is not universal among animals, and, given its potential absence from monotremes, it may not even be a shared feature of all mammals. New work by Smits et al. (2008) sheds light on the evolution of genomic imprinting through the identification of an imprinted locus for a noncoding RNA that is shared between placental mammals and marsupials. Previous efforts have only identified a handful of genes that are imprinted in both marsupials and placental mammals, and evidence suggested that the mechanisms of imprinting might be poorly conserved. Despite low sequence similarity, the authors were aided in their search for the marsupial homolog of the *H19* gene (known to be imprinted in placental mammals) by several characteristics. These include the proximity of *H19* to another imprinted locus (that of the *IGF2* gene), a shared exon structure with the *H19* gene of placental mammals, and the conservation of a microRNA (miR-675) in exon 1. In placental mammals, *H19* is only expressed from the maternal locus, and the same was shown to be true in tissues from young wallabies (still in the pouch). The authors also show that the paternal copy of wallaby *H19* is methylated upstream of the gene in a region containing binding sites for CTCF, a protein that insulates promoters from the effects of distant enhancers. In placental mammals, CTCF is the master regulator of imprinting in the *H19-IGF2* region, suggesting that the overall mechanism of imprinting is conserved between placental mammals and marsupials. Looking forward, it will be interesting to determine whether any of these elements of genomic imprinting are found in the more distantly related monotremes. Also, identifying the targets of miR-675 that are shared between marsupials and placental mammals might reveal reasons behind the conservation of this noncoding RNA.
G. Smits et al. (2008). Nat. Genet. Published online June 29, 2008. 10.1038/ng.168.

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